

Amendments to the Specification:

Please replace the paragraph on page 6, line 20, with the following amended paragraph:

Figure 1 is the amino acid sequence ~~(SEQ ID NO: 1)~~ (SEQ ID NO: 2) for residues 308 to 348 of human ZP3 ~~(SEQ ID NO: 1)~~.

Please replace the paragraph on page 6, lines 21-22, with the following amended paragraph:

Figure 2 is a representative prediction summary of O-glycosylation sites on the 308-348 amino acid region (SEQ ID NO: 2) of human ZP3 ~~(SEQ ID NO: 1)~~.

Please replace the paragraph on page 6, lines 23-24, with the following amended paragraph:

Figure 3 is a representative prediction summary of O-glycosylation sites on the 309-349 amino acid region (SEQ ID NO: 3) of mouse ZP3 ~~(SEQ ID NO: 2)~~.

Please replace the paragraph beginning on page 14, line 12, with the following amended paragraph:

According to preferred embodiments the DNA sequence useful for hZP3 transgenic expression codes for a shorter protein (polypeptide) molecule, (i.e. less than 25kDa polypeptide, particularly less than 10kDa polypeptide and more particularly less than 5 kDa). Such preferred protein sequences include a core region as shown in SEQ ID NO: 2. The inventors realized that SEQ ID NO: 2 ~~SEQ ID NO: 1~~ is the most important determinant with respect to the inter-species glycosylation pattern of ZP3 and that a small protein having this sequence, for a given mass, will be particularly potent in binding sperm. The SEQ ID NO: 2 is 54% homologous with the corresponding sequence from mouse ZP3. By "homologous" is meant that when the two sequences are compared, 54% of the amino acids are the same. Despite the homology, the

mouse ZP3 has a small amount of binding with human sperm, although this affinity is less than one-tenth, and typically less than one-hundredth the level observed with human ZP3. The term "can strongly bind human spermatozoa" is used herein to mean binding that is at least about 10 times (e.g. 10 times) as strong as an equivalent molar amount of mouse ZP3. In practice, such a qualitative determination is carried out by incubating different concentrations of material in a binding assay and determining binding directly or indirectly by competition. Such binding assays are exemplified in the examples herein and are well known to the skilled artisan. Thus, a peptide sequence that differs from human ZP3 by up to 46% maintains some residual human sperm binding activity that is stronger than the equivalent molar amount of mouse ZP3.

Accordingly, a peptide according to one embodiment is more than 46% identical to the human ZP3 sequence shown in SEQ ID NO: 2. Furthermore, it is preferred that any deviation from this sequence be limited to positions 3, 8, 13, 16, 17, 19, 21-23, 25, 27, 28, 30, 32-35, 38 and 39, (positions listed in Table Figure 1) as the non-listed positions are more conserved.

Please replace the paragraph on page 17, lines 20-29, with the following amended paragraph:

Both binding and acrosome reaction can be tested for detecting rhZP3 biological activity. For binding tests, rhZP3 is used in detecting the initial stage of binding sperm to zona pellucida. In other embodiments, the recognition and binding of a sperm sample to rhZP3 ~~hrZP3~~ is directly tested in solution, or after immobilization of rhZP3 ~~hrZP3~~ to a solid support such as SEPHADEX (hydroxypropylated, cross-linked dextran matrix or beads) ~~Sephadex (TM)~~ or an agarose resin. These functional tests evaluate the binding capacity of sperm, and can use (a) rhZP3 conjugated beads (solid phase) or, (b) as part of an ELISA-like test, rhZP3 free in solution (liquid phase). In the latter case, rhZP3 preferably is conjugated to another moiety that can form a signal in the assay. Sperm from infertile male individuals, and that lack ZP3 binding activity will not bind to rhZP3 and hence, can be diagnosed and identified.

Please replace the paragraph on page 19, lines 5-13, with the following amended paragraph:

A pair of primers (A and B primers) was designed from the reported sequence of hZP3. Chamberlin and Dean *supra*. Primer A is located at the 5' end of the hZP3 cDNA from bases 8 to 29 as ~~(SEQ ID NO: 4)~~ 5'ACCATGGAGCTGAGCTATAGG3' (SEQ ID NO: 4) ~~(SEQ ID NO: 3)~~. Primer B is located at the 3' end of the hZP3 cDNA from bases 1256 to 1282 as ~~(SEQ ID NO: 5)~~ 5'TTCTCGAGTTAATGATGATGATGATGATGATGATCGGAAGCAGACACAGGGTGGGAGGCAGT3' (SEQ ID NO: 5) ~~(SEQ ID NO: 4)~~. A sequence at an XhoI restriction site (CTCGAG) and a sequence coding for 6 histidine residues were added to the 3' end of primer B for the purpose of subcloning the cDNA into the expression vector as well as for purification of the hZP3 from the medium.

Please replace the paragraph on page 22, lines 7-23, with the following amended paragraph:

Histidine tagged glycoprotein (rhZP3) was purified from the glycoprotein fraction, and isolated from cultured cell media with WGA affinity chromatography, with Ni-NTA (nitrilo-tri-acetic acid) resin (Qiagen). Proteins containing one or more 6xHis affinity tags, located at either the amino or carboxyl terminates of the protein, bind to the Ni-NTA resin with an affinity ($K_d = 10^{-13}$ at pH 8.0) far greater than the affinity between most antibodies and antigens, or enzymes substrates ~~suspended~~ ~~uspended~~ 50% slurry of Ni-NTA resin were transferred into the column. Two milliliter of resin were completely resuspended then washed with 5 resin-volume of H₂O. The resin was equilibrated with 10 resin-volumes of Ni-NTA binding buffer. The WGA isolated glycoprotein samples that had been dialyzed with Ni-NTA binding buffer were passed through an equilibrated Ni-NTA column that contained 1 ml of resin. The flow rate was adjusted to 3-4 resin volumes per hour. After passing the glycoprotein sample through the Ni-NTA column, the resin was washed with 10 resin ~~columns~~ ~~columns~~ of Ni-NTA binding buffer that contained TWEEN-20 ~~Tween~~ (Fisher) (polyoxyethylene sorbitan monolaurate) and 2-mercaptoethanol, was

followed by a wash with Ni-NTA washing buffer (50 mM PBS, pH 6.6, 300 mM NaCL) until the flow-through 280nm absorbance was less than 0.01. His-tagged glycoproteins were eluted from the resin with the Ni-NTA washing buffer that contained 40 mM of imidazole (Sigma).